ON THE VARIATION AND INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *OPHRYOSCOLEX PURKYNJEI* (INFUSORIA OLIGOTRICHA).

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(With Two Text-figures.)

The investigations of Jennings (1916) and his pupils (Hegner, 1918, 1919, Middleton, 1915), as well as those of Jollos (1921, 1924), established the presence of many different stocks, pure lines or families in several species of Protozoa. These stocks may differ in their physiological functions (rate of fission, endurance and so on), or they are distinguished by morphological features. Examples of the former are found in Infusoria and of the latter in Rhizopoda. It must be noted that the characters concerned in Rhizopoda (*Difflugia, Arcella*) relate to the form and size of the shell, or to the size and number of different shell-outgrowths (spines, teeth). The mode of inheritance of these characters during uniparental reproduction was also established by the authors mentioned above. It was shown that for instance the number of spines may vary between the parent and its direct offspring. A doubt may arise however whether the characters studied belong to the shell and not to the protoplasmic body of the animal, being that is to say alloplasmatic structures. Although we do not think this objection serious, still it seems well worth strengthening the results of previous authors by investigations on truly protoplasmic morphological characters in Protozoa.

Some of the Ophryoscolecidae infesting in great numbers the stomach of oxen, sheep and other Ruminantia seem to be well adapted for such studies. They possess many very strongly developed morphological characters (for instance the number, size and mode of branching of caudal spines), facilitating the study of variation and inheritance. A form especially suited for such work is *Ophryoscolex purkynjei* with several circles of spines surrounding the posterior end of the body. These spines do not represent simple cuticular outgrowths but are of a very complex structure, consisting of ectoplasm with interior skeletal rods and contractile fibrils, the whole covered with a cuticular sheath. Therefore in

examining the variation and inheritance of these spines we have to do with a typical protoplasmic part of the body.

Two questions are dealt with in this paper: (1) can the species *O. purkynjei* be divided into several stocks differing in their morphological characters? as such characters we consider the number of branches in the forked spines of the first circle; (2) is the character of branching of spines retained or changed in daughter-individuals produced by fission from the same parent?

The most important points in the morphology of Ophryoscolex purkynjei.

This infusorian has a somewhat barrel-like body, 150–200μ long and 80–100μ wide. The anterior end of the body is occupied by a wide peristome, surrounded by a spirally disposed adoral zone of membranelles. Another set of membranelles (the dorsal zone) is situated on the dorsal side of the animal. This zone encircles not only the back but also the lateral sides of the body. It forms a sort of crescent, the membranelles being absent on the ventral side of the body only. The dorsal zone divides the body into two parts: anterior or prezonal, and posterior or postzonal. The prezonal part has the appearance of a truncated cone with a completely smooth surface. The postzonal part possesses on its dorsal and lateral sides seven shallow longitudinal grooves, as in the surface of a peeled orange. The ventral side of the postzonal part remains grooveless. These grooves divide the postzonal part of the body into seven longitudinal sections: the largest of them occupies the ventral side of the animal, while the remaining six (all of them nearly of the same width) take up the lateral sides of the infusorian and its back. We designate the six latero-dorsal sections as sections I–VI, numbering from left to right. Sections I–V contain the contractile vacuoles (nine in number); section VI has no vacuoles because it is occupied by a sausage-shaped macronucleus, underlying the cuticle.

The posterior end of the body is elaborately adorned with numerous spines whose distribution is very constant. The ventral section of the postzonal part gradually tapers backwards until it becomes a strong ventral spine protruding at the posterior end of the animal. The right side of the ventral spine is furnished at its base with a secondary spine. The remaining sections of the body bud off three circles of lesser spines. The first or anterior circle is formed by six large spines, bi-, tri- or quadri-furcated at their free end. All the branches or teeth of a spine lie in one plane, the spine having a flattened aspect. In very rare cases (three times in several hundred animals investigated) the number of

branches may rise to five. There may be differences in the mode of branching of the spines. In most cases all the branches of a spine are equally well developed. In other and rarer cases there are two or three equal branches, one of which is split in two or gives off a collateral branch, by which process the whole number of branches is increased to three or four. Every spine exhibits a very complex structure. It consists of an ectoplasmic outgrowth covered with a thin cuticle and supported

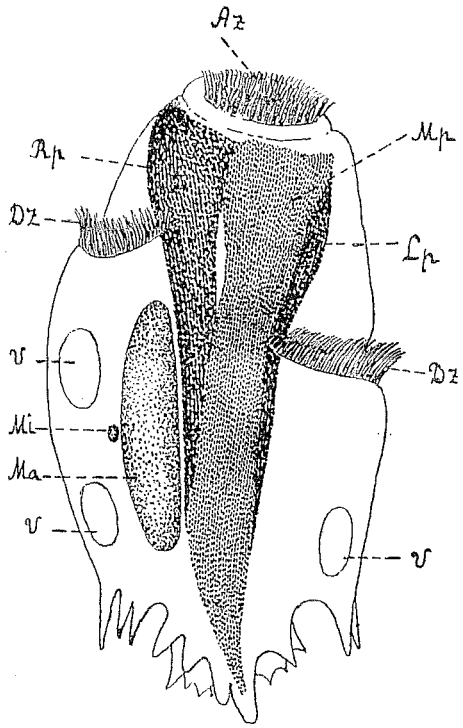


Fig. 1. *Ophryoscolex purkynjei* seen from the ventral side. *Az*, adoral zone of membranelles; *Dz*, dorsal zone of membranelles; *Lp*, *Mp*, *Rp*, left, ventral and right skeletal plates; *v*, contractile vacuoles; *Ma*, macronucleus; *Mi*, micronucleus.

by an inner skeletal frame. This latter has the form of a semicircular arc, occupying the base of the spine, from which diverge 2–4 thin skeletal rods entering the branches of the spine and penetrating to their free end. Moreover the ectoplasm of the spines contains a system of thin muscular fibrils or myonemata.

The second circle of spines is situated behind the first one and consists of about twenty (this number can vary) independent short spines

projecting from the border of a circular rim which surrounds the posterior end of the body. The third circle contains only 5–7 spines lying at the base of the large ventral spine just inside the second circle.

The present paper deals exclusively with the variation and inheritance of the number of branches in the spines of the first circle.

At the bottom of the peristome lies the mouth which opens into a strongly developed pharynx. The latter extends to the hinder part of the body, being closely applied to the right surface of the body. Between the pharynx and the body-wall are inserted three skeletal plates which support the right, the left and the ventral sides of the gullet. These plates consist of a substance nearly akin to the cellulose of plants and are vividly stained a dark violet by chloro-iodide of zinc, and blue by iodine and sulphuric acid. The right and the ventral plates fuse together and continue far into the base of the big ventral spine. For present purposes there is no need to describe further cytological details of *Ophryoscolex purkynjei*.

Variation of the number of branches in the spines of O. purkynjei.

On examining different specimens of the same population of *O. purkynjei* it is easy to notice that the number of branches in the spines of the first circle can vary to a great extent. The fundamental form (and the only one known heretofore from the descriptions of Eberlein and others) of the spines appears to be a three-branched one. This ground-plan is in many specimens retained by the majority of the spines. Sometimes (but not very often) all the spines of a specimen are three-branched. Yet usually some of the spines exhibit a plus- or minus-variation, producing two, four or (very seldom) even five branches. In different specimens of the same population the number and the position of the two- or four-branched spines may vary. So for instance we have the following differences in 10 specimens taken at random from the population No. 248.

Number of branches in	Specimen No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
Spine No. I	4	3	4	4	3	4	3	4	3	3
Spine No. II	4	2	3	3	3	3	3	3	2	2
Spine No. III	3	2	2	3	2	3	2	3	2	2
Spine No. IV	3	3	3	3	3	3	3	3	2	3
Spine No. V	3	2	3	3	3	3	3	3	4	3
Spine No. VI	3	3	3	3	3	3	3	3	3	3

Still there always exists in the same population a tendency in some spines to augment and in others to reduce the number of their branches. So for instance in the population No. 43 the second spine in 36 cases out

of 50 has only two branches, while the first spine possesses two branches only in 2 specimens out of 50. Or, to take another case, in the population No. 248 the first spine possesses four branches in 22 specimens out of 50, while the spines III and IV exhibit the same feature only in 1 specimen out of 50.

The study of populations taken from different specimens of the host has shown that they differ in their mode of variation, each population possessing its own characteristic features. It can be best exemplified by a comparison of several populations tabulated below. Table I is prepared after an examination of six sets of 50 specimens each, taken from different populations (Nos. 43, 242, 248, 250, 254 and 260).

TABLE I.

Showing the character of branching in six different populations of O. purkynjei. *For every population are examined* 50 *specimens taken at random.*

	Absolute number of spines						The same in percentages					
	Population No. 43	Population No. 250	Population No. 260	Population No. 254	Population No. 248	Population No. 242	Population No. 43	Population No. 250	Population No. 260	Population No. 254	Population No. 248	Population No. 242
Spine I												
Bifurcated	1	2	1	—	—	—	2	4	2	—	—	—
Trifurcated	46	35	38	35	28	31	92	70	76	70	56	62
Quadrifurcated	3	13	11	15	22	19	6	26	22	30	44	38
Spine II												
Bifurcated	36	27	4	4	14	2	72	54	8	8	28	4
Trifurcated	14	20	42	38	33	42	28	40	84	76	66	84
Quadrifurcated	—	3	4	8	3	6	—	6	8	16	6	12
Spine III												
Bifurcated	39	32	12	13	14	7	78	64	24	26	28	14
Trifurcated	11	17	35	36	35	39	22	34	70	72	70	78
Quadrifurcated	—	1	3	1	1	4	—	2	6	2	2	8
Spine IV												
Bifurcated	35	17	10	11	5	6	70	34	20	22	10	12
Trifurcated	15	33	36	34	44	43	30	66	72	68	88	86
Quadrifurcated	—	—	4	5	1	1	—	—	8	10	2	2
Spine V												
Bifurcated	22	8	9	5	1	3	44	16	18	10	2	6
Trifurcated	27	41	39	39	43	36	54	82	78	78	86	72
Quadrifurcated	1	1	2	6	6	11	2	2	4	12	12	22
Spine VI												
Bifurcated	13	6	4	6	1	1	26	12	8	12	2	2
Trifurcated	34	38	45	41	40	37	68	76	90	82	80	74
Quadrifurcated	3	6	1	3	9	12	6	12	2	6	18	24
All the spines												
Bifurcated	147	92	40	39	35	19	49	31	14	13	12	6
Trifurcated	146	184	235	223	223	228	49	61	78	74	74	76
Quadrifurcated	7	24	25	38	42	53	2	8	8	13	14	18
Total of spines	300	300	300	300	300	300	100	100	100	100	100	100
Total of branches	756	832	885	899	907	934						

It is clear that these six populations can be ranged in two groups. One of the groups is characterized by a tendency to a minus-variation. Indeed in the populations Nos. 250 and 43 a great number (from 31 per cent. to 49 per cent.) of spines are bifurcated. Another group has no such marked tendency to bifurcation, but on the contrary may exhibit a large number of quadrifurcated spines. So in the populations 242, 248, 254 and 260 there are only 6–14 per cent. of bifurcated spines, while 8–18 per cent. of the spines become quadrifurcated, whereas populations 250 and 43 exhibit respectively 8 and 2·5 per cent. of quadrifurcated spines.

These facts seem to show definitely: (1) that there exist many races or pure strains of *O. purkynjei* differing in morphological characters; (2) that the characters named vary to a certain degree in the members of the same population.

Some of the spines have in all the populations an unquestionably stronger propensity to a plus- or minus-variation than the others. So for instance spine I has a marked tendency to increase the number of its branches; even in populations characterized by a preponderance of minus-variations in other spines spine I has more branches than the remaining ones. In the six thoroughly studied populations spine I was quadrifurcated in 6 per cent. (population No. 43), 22 per cent. (No. 260), 26 per cent. (No. 250), 30 per cent. (No. 254), 38 per cent. (No. 242) and 44 per cent. (No. 248) of all the specimens. Meanwhile a bifurcated spine I was found in three of the populations only, and then only in 2 per cent. (Nos. 43 and 260) to 4 per cent. (No. 250) of all the specimens examined. Or if we put together the results for all the populations the mean percentage of quadrifurcated first spines is 28 per cent., while that of bifurcated is 1·3 per cent. only.

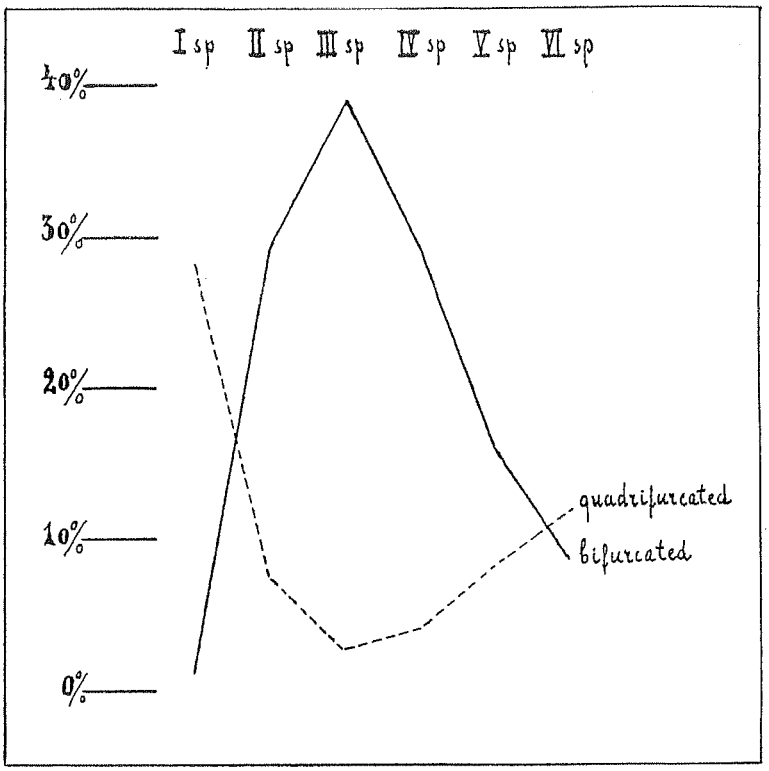
Let us take for comparison spine III which has the tendency to form minus-variations. It is quadrifurcated in 2–8 per cent. of all the cases; bifurcated III spines are found in 14 per cent. (population No. 242), 24 per cent. (No. 260), 26 per cent. (No. 254), 28 per cent. (No. 248), 64 per cent. (No. 250) and 78 per cent. (No. 43) of the whole population. The mean for six populations is: 3·3 per cent. of quadrifurcated and 39 per cent. of bifurcated III spines.

Comparing the data from 11 different populations of *O. purkynjei* for different spines of the first circle we remark that the disposition to minus-variations is most strongly developed in spine III (39 per cent. of bifurcated spines); from here this tendency decreases in both directions, *i.e.* towards spine I as well as towards spine VI (see Table II).

Plus-variations are most frequently met with in spine I; then their number diminishes in spine II and reaches its minimum in spine III; from here on the number of quadrifurcated spines again increases towards spine VI.

There is a reason why spine III exhibits such a slight tendency to increase the number of its branches. As we shall show in our

TABLE II.



detailed monograph of the Ophryoscolecidae, the nearest but more primitive allies of *O. purkynjei* possess only five bifurcated spines (I–V). Spine VI is a character newly acquired in this species, as well as in *O. caudatus*. Now in the above-mentioned more primitive species of *Ophryoscolex* (for instance in *O. diplospinosus* Dog. and Fed.) spine III corresponds to the median dorsal line of the body, lying just opposite to the big ventral spine. The latter (as we have seen in *O. purkynjei*) remains always unbranched, which character is also for a

long period preserved by its dorsal partner. On both sides of the dorso-ventral plane the tendency to form branches gradually increases in the ventral direction reaching its height in spine I and in (the newly acquired) spine VI.

The differences in the mode of branching in different populations are well shown if we try to compare the whole number of branches existing on the spines of the same number of specimens taken from different populations. Let us take lots of 50 individuals each from the six populations already mentioned above. Each lot possesses $50 \times 6 = 300$ spines. These 300 spines have on the whole in different populations the following number of branches:

Population No.	43—756	branches.	The	average	number of branches on a spine is	2·50
No.	250—832	,,	,,	,,	,, ,,	2·77
No.	260—885	,,	,,	,,	,, ,,	2·95
No.	254—899	,,	,,	,,	,, ,,	3·00
No.	248—907	,,	,,	,,	,, ,,	3·00
No.	242—934	,,	,,	,,	,, ,,	3·11

It is easy to notice that the difference between two populations (for instance Nos. 43 and 242) can be very considerable.

Inheritance of the number of branches in the spines of O. purkynjei *during the asexual reproduction.*

The reproduction is effected by a process of binary fission. The animal becomes constricted transversely on the level between the two rows of contractile vacuoles and gradually divides into an anterior and a posterior daughter-individual (Fig. 2). The posterior one gets the spine-armature of the parent, while the anterior forms the spines anew.

The branching of the new spines of the first circle begins before the separation of the daughter-individuals so that it is possible to compare the character of spines and their mode of branching in both the individuals. In this way one can judge if the character of branching in the parent is retained by the anterior daughter-individual. It is difficult to get numerous specimens in the latest stages of fission, but still we succeeded in collecting three lots of such individuals from different populations (Nos. 250, 254 and 260). One of the lots (No. 260) contained 20 specimens, while the other two (Nos. 250 and 254) were of 15 specimens each.

The first result of the investigation was that the newly arising spine of the anterior daughter individual may often differ in the number of

its branches from the corresponding old spine of the posterior individual. This difference can manifest itself in an increase as well as in a decrease in the number of branches in the new spine. One and the same individual can exhibit in different spines both these processes.

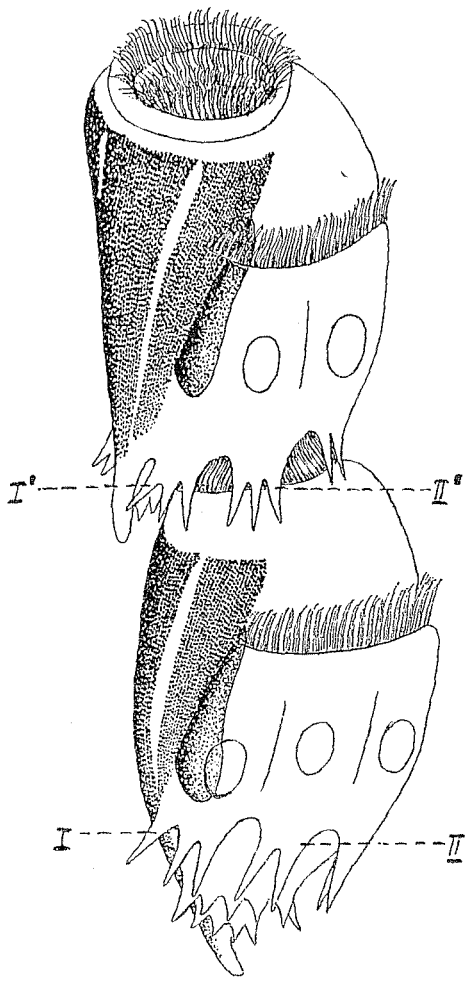


Fig. 2. *Ophryoscolex purkynjei* in a late stage of fission; *I* and *II*, first and second branched spines of the posterior daughter-individual; *I'* and *II'*, first and second branched spines of the anterior individual.

So for instance specimen No. 14 from the population 250 had the following spine-formula for the posterior (below the horizontal line) and anterior (above the horizontal line) daughter-individual:

Spines					
I	II	III	IV	V	VI
3	2	**3**	4	**3**	3
3	3	3	3	3	3

Such instances are by no means rare. On the contrary the number of specimens where the character of branching in both daughter-individuals completely coincides is very small. Out of the whole number of 50 specimens examined there were seven such fission-stages (or about 15 per cent.). Very often (see Table III) the anterior individual of an *Ophryoscolex* in a state of fission differs from the posterior one in the mode of branching of one of the spines only. Less often the difference was observed in two, three, or once even in as many as four of the spines.

TABLE III.

To show the inheritance of the branching of spines in the dividing specimens of the lot No. 254.

The lot contains 15 *specimens.*

Daughter-individ.		Spines I	I	III	IV	V	VI
No. 1	Anterior	4	3	3	**3**	3	**3**
	Posterior	4	3	3	2	3	3
No. 2	Anterior	3	3	**3**	3	4	3
	Posterior	3	3	2	3	4	3
No. 3	Anterior	**3**	3	3	3	**3**	**3**
	Posterior	**4**	3	3	3	**3**	**3**
No. 4	Anterior	**3**	**3**	**2**	**3**	**3**	**3**
	Posterior	4	3	3	4	3	3
No. 5	Anterior	3	3	3	3	3	**2**
	Posterior	3	3	3	3	3	3
No. 6	Anterior	3	3	3	**2**	3	3
	Posterior	3	3	3	3	2	3
No. 7	Anterior	3	3	**2**	3	3	**2**
	Posterior	3	3	3	3	3	2
No. 8	Anterior	**4**	3	3	3	3	3
	Posterior	3	3	3	3	3	3

Daughter-individ.		Spines I	II	III	IV	V	VI
No. 9	Anterior	**4**	3	3	**3**	3	3
	Posterior	3	3	3	3	3	3
No. 10	Anterior	**3**	3	3	3	3	**3**
	Posterior	4	3	3	4	4	3
No. 11	Anterior	3	**4**	**4**	**3**	3	3
	Posterior	3	3	3	**3**	3	3
No. 12	Anterior	3	3	3	**3**	3	**2**
	Posterior	4	4	3	3	3	2
No. 13	Anterior	**3**	3	3	3	4	**2**
	Posterior	4	4	3	3	4	3
No. 14	Anterior	**3**	3	3	**2**	3	3
	Posterior	4	3	3	3	3	3
No. 15	Anterior	3	3	2	3	**3**	3
	Posterior	3	3	3	2	4	3

For the 20 cases from population No. 260 the total number of spines dealt with belonging to the anterior individuals was $6 \times 20 = 120$, and for the 15 cases from populations No. 250 and 254 respectively was $6 \times 15 = 90$. Amongst these there were in the lot No. 260, 26 spines (or about 21·6 per cent.) differing from the spines of the posterior individual. In the lot No. 250 there were 24 such spines (or 26 per cent.), while the lot No. 254 possessed 27 such modified spines (or 30 per cent. of the whole number). Therefore at every fission the number of branches

is changed in about 20–30 per cent. of spines of the anterior daughter-individuals.

Is the disposition to vary expressed in all the spines in the same degree? The number of the specimens investigated is too small to permit us to solve this question. Still if we may judge from our scanty material the most variable of the spines are III and IV. In these spines the

TABLE IV.

	15 specimens of the Population No. 250		15 specimens of the Population No. 254		20 specimens of the Population No. 260	
	Posterior daughter-individ.	Anterior daughter-individ.	Posterior daughter-individ.	Anterior daughter-individ.	Posterior daughter-individ.	Anterior daughter-individ.
Spine I						
Bifurcated	—	—	—	—	1	1
Trifurcated	9	10	12	8	14	17
Quadrifurcated	6	5	3	7	5	2
Spine II						
Bifurcated	5	7	—	—	—	1
Trifurcated	9	7	14	13	20	18
Quadrifurcated	1	1	1	2	—	1
Spine III						
Bifurcated	5	7	3	1	3	4
Trifurcated	9	8	11	14	16	16
Quadrifurcated	1	—	1	—	1	—
Spine IV						
Bifurcated	2	4	2	2	1	4
Trifurcated	11	11	13	11	18	13
Quadrifurcated	2	—	—	2	1	3
Spine V						
Bifurcated	2	—	—	1	3	3
Trifurcated	12	14	13	10	16	16
Quadrifurcated	1	1	2	4	1	1
Spine VI						
Bifurcated	—	1	4	2	2	3
Trifurcated	13	13	11	13	17	16
Quadrifurcated	2	1	—	—	1	1
All the spines together						
Bifurcated	14	19	9	6	10	16
Trifurcated	63	63	75	69	101	96
Quadrifurcated	13	8	6	15	9	8
Total of spines	90	90	90	90	120	120
Total of branches	269	259	267	279	359	352

number of branches was changed in 34 and 36 per cent. respectively of the anterior daughter-individuals. Spine VI proved to be the most constant of the spines, as it altered the number of its branches in 14 per cent. of the individuals only. It is noteworthy that the newly acquired spine VI is less variable than the rest of the spines which are of more ancient origin.

Our data are too few to enable us to elucidate the question whether

the general character of a population is on the whole maintained throughout the life of the said population in the same host; or whether the character of branching may change in the course of generations arising by asexual reproduction. In the three lots examined the number of trifurcated spines was slightly increased in the anterior individuals in comparison with the posterior ones.

The high degree of variation in the number of spine-branches must perhaps be attributed to the following circumstance. The comparison of *O. purkynjei* with several other species closely related to it proves that *O. purkynjei* represents a species which has not yet attained the state of stable equilibrium. This species is on the way to increase the number of the branches in the spines of the first circle from two to four.

Summary.

1. The branched spines of *O. purkynjei* represent very complicated protoplasmic structures.

2. The number of branches in the spines of *O. purkynjei* varies from 2–4 in different members of the same population and in different spines of the same individual.

3. Every population possesses a somewhat different character of branching, which circumstance proves the existence of several different strains or races in the species named.

4. Spine I possesses in the highest degree a tendency to form plus-variations, while spine III is characterized by its disposition to minus-variations. This character remains true for all the populations examined.

5. The examination of 50 individuals in the late stages of fission proves that the newly arising spines of anterior daughter-individuals may very often differ in the number of branches from the corresponding old spines of posterior daughter-individuals. This difference manifests itself in an increased, as well as in a decreased, number of branches in the new spines.